Research Article

Sensitivity and specificity of Enzyme-Linked Immunosorbent Assays in Brucellosis

Zahra Soleimani[1], Reza Razzaghi[1], Alireza Sharif[1]* and Kataneh Khamooshi[2]

[1]Infectious Diseases Research Center, Kashan University of Medical Sciences, Kashan, Iran
[2]Student Research Committee, Kashan University of Medical Sciences, Kashan, Iran

DOI: 10.5455/jrmds.2016444
Corresponding Authors Email: arsharifmd@yahoo.com
Received on: 02/08/2016
Accepted on: 28/11/2016

ABSTRACT

Background: Brucellosis is a public health problem in the world. Several diagnostic tests have been proposed for the diagnosis of brucellosis, including Standard Agglutination Test (SAT) and Coombs anti-brucella tests. However these tests does not discriminate the immunoglobulin classes of this condition. The aim of current study was to explore the sensitivity and specificity of Immunosorbent Assay (ELISA) IgG and IgM tests in patients with Brucella bacteremia. **Material and Methods:** Thirty-one patients with clinical features suggestive of Brucellosis and were positive blood cultures for Brucella species, were enrolled. Brucella enzyme linked immunosorbent assay test determining the Brucella specific IgG and IgM, antibodies was utilized for the diagnosis of Brucellosis. **Results:** 31 patients including 18male (52.2%) and 13 female (47.8%) were used for IgG and IgM tests. 23 subjects had a history of dairy consumption. In 6subjects (20.3%) the history of brucellosis in family was positive.2 subjects had contact with cattles. Our data showed that the sensitivity, specificity, PVP and PVN of IgMwere100, 72.7,100 and 83.3%, while these values in IgG test were 100, 63.3, 86.9, 100% respectively. **Conclusion:** Our findings further confirmed that diagnostic value of ELISA based IgG and IgM with high sensitivity, compared to agglutination test, suggesting its value as a simple, rapid, sensitive and reliable method for the diagnosis of brucellosis.

Keywords: ELISA, IgG, IgM, Brucellosis

INTRODUCTION

Brucellosis is found to be globally distributed and leads to zoonotic disease [1, 2]. 500,000 cases of Brucellosis in the world are reported via World Health Organization [2-4]. The incidence of brucellosis in the United States is less than 5 cases per 100,000 populations [4]. The prevalence of brucellosis in humans and animals are increasing in Mediterranean countries, the Middle East, West Asia and Africa [5]. In Iran, Brucellosis experienced a descending trend from 1989 to 1996, and increased from 90000 cases in 1996 to 16000 cases in1996 [6]. Brucellosis can be characterized by several complications, such as endocarditic, meningitis, encephalitis, arthritis, bursitis, osteomyelitis, tenosynovitis, sacroileitis, psychiatric, pneumonia and hepatitis symptoms [1]. Agglutination test is being used for the diagnosis of this condition (Wright and Coombs Wright 2ME standard tube approach (STA)). Recently, anti-Brucella IgG and IgM tests are considered due to its simplicity [7] and being suggested as a potential diagnostic test. In a study in Saudi Arabia, The sensitivity and specificity of agglutination test were reported to be 100% and 1.79%, respectively [8]. Another study in Turkey population showed that IgG, IgM based ELISA is an appropriate test with sensitivity and specificity of 99% and 100%, respectively [9]. According study in Serra found the sensitivity and specificity of 99 and 8.98%, respectively, for this potential test, compared the agglutination [10]. Mohrez et al. evaluated this test in a small population of Iran in 2000. They detected the sensitivity and specificity of around 93 and 100%, respectively in the diagnosis of brucellosis [7].Therefore in the present study we further evaluated the potential of this test, compared with the agglutination immunoassay diagnostic test in

patients infected with brucellosis admitted to the clinic in Kashan, Iran, in 2015.

MATERIALS AND METHODS

In the current study, blood samples were obtained from patients infected by brucellosis. The criterion for a positive test IgM and IgG was higher than 10 and as for Wright and Coombs test was around80.1, while this value for 2ME was 40.1. 31 patients suspected of having brucellosis were enrolled. The patients did not affected by tuberculosis, pernicious anemia, tularemia and cholera, which were false agglutination test positive. IgG, IgM based ELISA using DRG kit was used according to the manufactory's protocol (Germany). Data were plotted as frequency distribution value followed by the analysis of sensitivity, specificity, positive and negative predictive values. All the analyses were two-sided and statistical significance was set at $P<0.05$. Informed consent was obtained from all participants using protocols approved by the Ethics Committee of Kashan University of Medical Sciences.

RESULTS

In the present study 31patients suspected of having brucellosis with mean age of 8.41 ± 2.17 were studied. Their gender composition included 18 males and 13 females. The results showed that 20 patients were positive for agglutination test. Moreover, the results of IgG and IgG tests were positive in 24 and 23 patients, respectively. Additionally we observed that the sensitivity and specificity of IgM test was 100 and 63.3%, while these values for IgG test were 100% and 72.7%, respectively. Negative and positive predictive values were estimated about 100 and 3.83% in IgM test, however these values for IgG tests were 9.86 and 100%, respectively.

Table 1. Frequency distribution of ELISA (IgM) test results according to standard agglutination test in study subjects

ELISA (IgM) agglutination	ELISA (IgM)		Sum
	Positive	Negative	
Positive	(83.3)20	(0)0	(64.5)20
Negative	(13.1)3	(100)8	(35.5)11
Sum	(100)23	(100)8	(100)31

Table 2. Frequency distribution of ELISA (IgM) test results according to standard agglutination test in study subjects

ELISA(Ig G) agglutination	ELISA (IgG)		Sum
	Positive	Negative	
Positive	(86.9)20	(0)0	(64.5)20
Negative	(16.7)4	(100)7	(35.5)11
Sum	(100)24	(100)7	(100)31

Table 3.The value criteria of ELISA (IgG&IgM) tests in diagnosis of brucellosis patients

Diagnosis Tests	Variations			
	Sensivity	Specificity	Predictive Value positive	Predictive Value Negative
ELISA(IgM)	(100)20	(63.3)7	(83.3)20	(100)7
ELISA(IgG)	(100)20	(72.7)8	(86.9)20	(100)8

DISCUSSION

We demonstrated that the sensitivity of IgG and IgM based ELISA tests was about 100% and their specificities were 72.7 and 63.3% respectively. The positive predictive values of both tests were86.9% and83.3% for IgG and IgM, respectively, suggesting the value of this test for detection of brucellosis.

Several serological tests are being recognized in the diagnosis of brucellosis. The most widely used is Standard Tube Agglutination and Coombs anti-brucella tests. Different studies have evaluated the diagnostic value of IgG and IgM tests, including Serra (2004) in Spain, which they proposed the sensitivity and specificity of this test with more than 98% and its being recommended as an adjunctive test (10). Ciftci and colleagues showed that the sensitivity and specificity of this test was 71.4% and 94.3%, respectively [11]. Several other studies have reported the diagnostic potential of this test, compared to other serologic tests [12]. In particular Mathai et al., in 1996 demonstrated its value, compared to standard agglutination [13]. Another

study by Calvijo and collaborators revealed the higher sensitivity in the study Dipstick and agglutination test [14]. Memish et al., found that the sensitivity and specificity of agglutination test was 6.95% and 100%, respectively, while these values in IgM/IgG was 6.45 and 1.97, respectively [8].

In conclusion we study further confirm the diagnostic value of IgM and IgG test for detection of brucellosis. Our findings showed that the sensitivity of these new tests was much more than agglutination test and can be used as a diagnostic test for brucellosis. Although further studies in a larger and multi center setting are needed to explore its values.

CONCLUSION

Our findings further confirmed that diagnostic value of ELISA based IgG and IgM with high sensitivity, compared to agglutination test, suggesting its value as a simple, rapid, sensitive and reliable method for the diagnosis of brucellosis.

Acknowledgments

This paper is derived from the residency dissertation (No: 91101) of Kataneh Khamooshi and its cost has been paid from the authorized protocol of Vice Chancellor of Research and Technology in Kashan University of Medical Sciences.

Funding source

Vice Chancellor of Research and Technology in Kashan University of Medical Sciences

Conflict of Interest

The authors have no conflict of interest to disclose.

Authors' Contribution

Zahra Soleimani developed the study concept and design and the acquisition of data, interpretations of data, and drafting of the manuscript. Reza Razzaghi, Alireza Sharif and Kataneh Khamooshi developed the protocol, analysis of data and drafting of the manuscript.

REFERENCES

1. Bennett JE, Dolin R, Blaser MJ. Mandell, Douglas, and Bennett's Principles and Practice of Infectious Diseases. 8th Edition. Philadelphia: Churchill Livingstone Elsevier; 2015. :2584-2589.
2. Ghomashlooyan M, Azami M, Mohaghegh MA, Hadipour M, Jahani S, Hejazi SH, et al. Evaluation of Isfahan Inhabitant Attitude About Brucellosis and Common its Herbal Treatment. International Journal of Infection. 2015;2(3).
3. Xiao P, Yang H, Di D, Piao D, Zhang Q, Hao R, et al. Genotyping of Human Brucella melitensis Biovar 3 Isolated from Shanxi Province in China by MLVA16 and HOOF. PloS one. 2015;10(1).
4. Assenga JA, Matemba LE, Muller SK, Malakalinga JJ, Kazwala RR. Epidemiology of Brucella infection in the human, livestock and wildlife interface in the Katavi-Rukwa ecosystem, Tanzania. BMC veterinary research. 2015;11(1):189.
5. Masters BR. Mandell, Douglas, and Bennett's Principles and Practice of Infectious Diseases, (2015) Eds: John E. Bennett, Raphael Dolin, Martin J. Blaser. ISBN: 13-978-1-4557-4801-3, Elsevier Saunders. Graefe's Archive for Clinical and Experimental Ophthalmology. 2015;1-3.
6. Abdon A, editor Brucellosis in the eastern Mediterranean Health region. proceeding on the regional conference on emerging infectious disease cario-Egypt; 1995.
7. Mohraz M, Kariminia A, Sarafnejad A. The Evaluation of Serologic Test (Elisa) in Brucellosis identification at Imam Khomaini Hospital. Iran Journal of infectious and tropical disease. 2003;8(23):10-3.
8. Memish Z, Almuneef M, Mah M, Qassem L, Osoba A. Comparison of the Brucella Standard Agglutination Test with the ELISA IgG and IgM in patients with Brucella bacteremia. Diagnostic microbiology and infectious disease. 2002;44(2):129-32.
9. El-Rab MG, Kambal A. Evaluation of a brucelle enzyme immunoassay test (ELISA) in comparison with bacteriological culture and agglutination. Journal of Infection. 1998;36(2):197-201.
10. Serra J, Viñas M. Laboratory diagnosis of brucellosis in a rural endemic area in northeastern Spain. International Microbiology. 2004;7(1):53-58.
11. Ciftci C, Oztürk F, Oztekin A, Karaoğlan H, Saba R, Gültekin M, et al. [Comparison of the serological tests used for the laboratory diagnosis of brucellosis]. Mikrobiyoloji bulteni. 2005;39(3):291-29.
12. Araj GF. Update on laboratory diagnosis of human brucellosis. International journal of antimicrobial agents. 2010;36:S12-S7.

13. Mathai E, Singhal A, Verghese S, D'Lima D, Mathai D, Ganesh A, et al. Evaluation of an ELISA for the diagnosis of brucellosis. The Indian journal of medical research. 1996;103:323-324.
14. Clavijo E, Díaz R, Anguita A, García A, Pinedo A, Smits HL. Comparison of a dipstick assay for detection of Brucella-specific immunoglobulin M antibodies with other tests for serodiagnosis of human brucellosis. Clinical and diagnostic laboratory immunology. 2003;10(4):612-615.